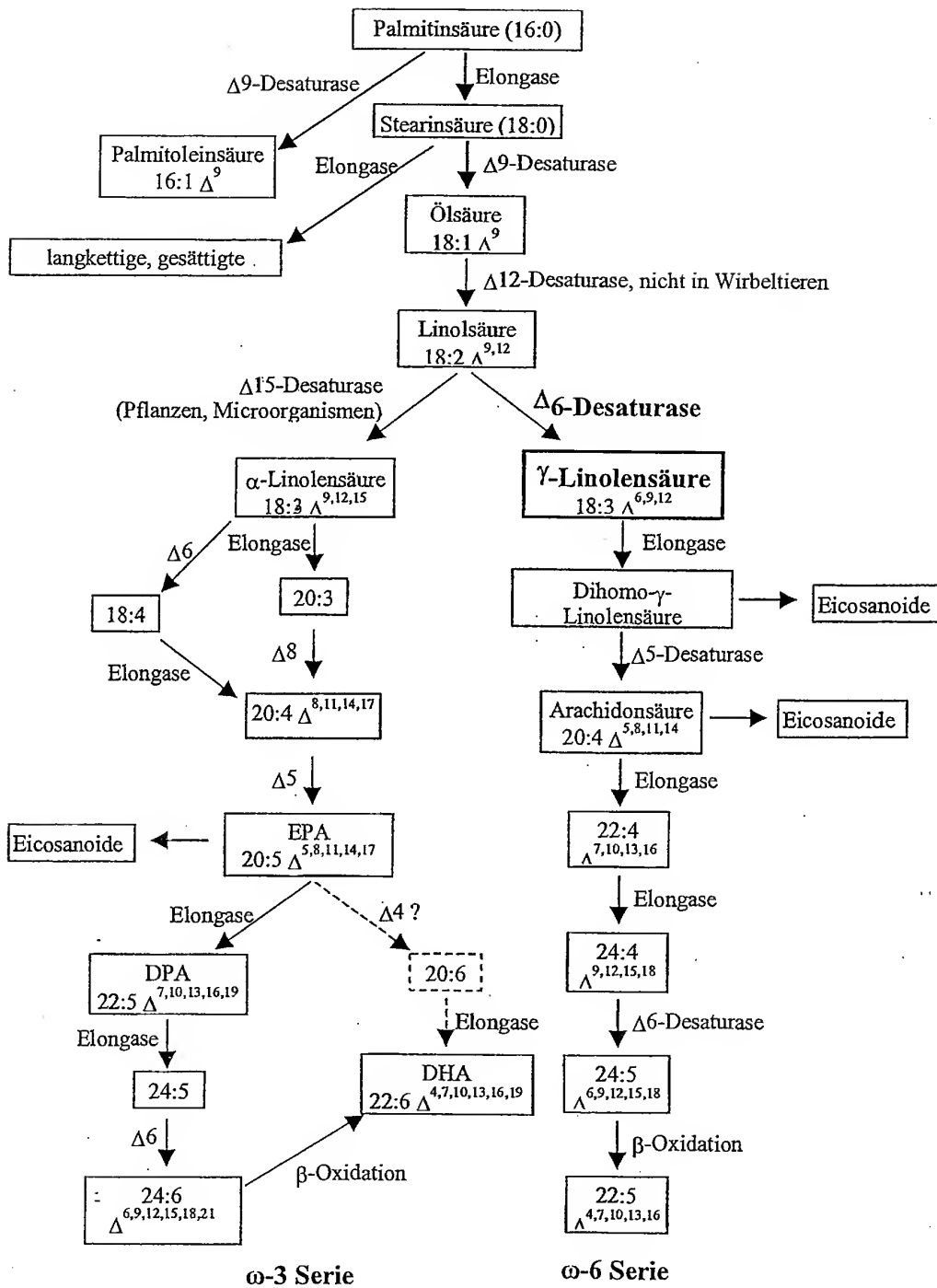


Figure 1



## Figure 2

BLASTP 2.0.8 [Jan-05-1999]

Reference: Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

Query= T.thermophila, delta-6-Desaturase (352 letters)

Database: /LION/data/db/fast/nrdb

387,705 sequences; 119,829,732 total letters

Sequences producing significant alignments:	Score	E
	(bits)	Value
trembl AF078796 AF078796_1 gene: "des-5"; product: "delta 5 fat...	79	4e-14
trembl AF031477 AF031477_1 product: "delta6-fatty-acid-desatura...	79	4e-14
trembl Z81122 CET13F2_1 gene: "T13F2.1"; Caenorhabditis elegan...	78	1e-13
trembl Z70271 CEW08D2_2 gene: "W08D2.4"; Caenorhabditis elegan...	78	1e-13
trembl AF005096 AF005096_1 product: "desaturase/cytochrome b5 p...	70	3e-11
trembl AJ222980 PPAJ2980_1 gene: "des6"; product: "delta6-acyl-...	69	6e-11
trembl U79010 BOU79010_1 product: "delta 6 desaturase"; Borago...	67	2e-10
trembl AC005397 AC005397_14 gene: "T3F17.14"; product: "putativ...	67	2e-10
trembl AF007561 AF007561_1 product: "delta-6-Desaturase"; Bora...	66	4e-10
tremblnew AF126799 AF126799_1 product: "delta-6 fatty acid desa...	64	2e-09
tremblnew AF126798 AF126798_1 product: "delta-6 fatty acid desa...	63	3e-09
trembl AF031194 AF031194_1 gene: "S276"; product: "S276"; Trit...	62	6e-09
tremblnew AB021980 AB021980_1 product: "delta-6 fatty acid desa...	62	6e-09
tremblnew AL078610 SCH35_12 gene: "SCH35.42c"; product: "putati...	62	8e-09
trembl AJ224160 BNAJ4160_1 gene: "sld1"; product: "delta-8 sphi...	60	2e-08
trembl AC004770 AC004770_2 product: "BC269730_2"; Homo sapiens...	60	3e-08
trembl AJ224161 ATAJ4161_1 gene: "sld1"; product: "delta-8 sphi...	59	6e-08
tremblnew AL050118 HSM800210_1 gene: "DKFZp586C201"; product: "...	57	2e-07
trembl AB022097 AB022097_1 product: "delta 5 fatty acid desatur...	57	2e-07
trembl X87143 HACYTB5RN_1 product: "cytochrome b5 containing fu...	50	2e-05
trembl Y08460 MMDES_1 gene: "Mdes"; product: "Mdes protein"; ...	50	3e-05
trembl AF001394 AF001394_1 product: "fatty acid desaturase/cyto...	46	4e-04
trembl AF002668 HSAF2668_1 product: "MLD"; Homo sapiens putati...	46	5e-04
swiss Q08871 LLCD_SYNY3 LINOLEOYL-COA DESATURASE (EC 1.14.99.25...	43	0.003

### Figure 3A

>aageneseq|W95504|W95504 Mortierella alpina delta 6 desaturase. Length = 457

Score = 89.7 bits (219), Expect = 4e-18

Identities = 102/422 (24%), Positives = 152/422 (35%), Gaps = 88/422 (20%)

```

Query: 9  EIVLENKPPELLNEYKFIYKDTEYDCTEYAKSNKHPGGLNFLNLFIDEKQDLTEYFRTLHS 68
      E + E K +      + I + YD E+      HPGG L      +D T+ F T H
Sbjct: 19  EALNEGKKDAEAPFLMIIDNKVYDVREFVPD--HPGGSVILT---HVGKDGTDVFDTFHP 73

Query: 69  KQALKILKSFPKTGAKQEETE-SSKRFSILKKLKHLEFPNWPPIEG----LFLTTFTLF 123
      + A + L +F      + + + + F+      +KL+ LF+      +      F +F L
Sbjct: 74  EAAWETLANFYVGDI DESDRDIKNDFAAEVRKLRTLFQSLGYDSSKAYYAFKVSFNL 133

Query: 124  VTGCLT---QKW-----YFSIPLLVLQMIIISGWIGHSMNHNRPILR----KFALVY 168
      + G T      KW      S LL L      GW+ H      H++      R      F
Sbjct: 134  IWGLSTVIVAKWGQTSTLANVLSAALLGLFWQQCGWLAHDFLHHQVFQDRFWGDLFGAFL 193

Query: 169  APLCGGFSNKKWGRKHNQHMFNTNNILKDEDIQ-HDYKLWQ----- 208
      +C GFS+ WW      KHN HH      N      +D DI H      W
Sbjct: 194  GGVCQGFSSSWWKDKHNTHHAAPNVHGEDPDIDTHPLLTWSEHALEMFSDPDEELTRMW 253

Query: 209  -----FP---FLFLKWKLDLSIL-----ASYEFEGIFLALHWV 238
      FP      F L W L SIL      E + LA+HW
Sbjct: 254  SRFMVLNQTFWYFPILSFARLSWCLQSILFVLPNGQAHKPSGARVPISLVEQLSLAMHWT 313

Query: 239  LLFNQNFYIV-----ILSELIAGFFSASILVGNHEN--EMKFERRITLPFFEHQI 286
      F +      ++S+ + G A + NH      + E + + FF QI
Sbjct: 314  WYLATMFLFIKDPVNMLVYFLVSQAVCGNLLAIVFSLNHNMGMPVISKEEAVDMDFFTQI 373

Query: 287  AASRNYAFHDIFSLIMGGMQYQTEHHFFPQIPFYRLPKARVIIAEELKKWNLKIHEGPI 346
      R+      +F+      GG+ YQ EHH FP +P + K + +      KK+N++ H +
Sbjct: 374  ITGRD-VHPGLFANWFTGGLNYQIEHHLFPSMPRHNFPSKIQPAVETLCKKYNVRYHTTGM 432

Query: 347  FE 348
      E
Sbjct: 433  IE 434

```

Figure 3B

>trembl|AF031477|AF031477\_1 product: "delta6-fatty-acid-desaturase";  
Caenorhabditis elegans delta6-fatty-acid-desaturase mRNA, complete cds.  
//:gp|AF031477|3088520 product: "delta6-fatty-acid-desaturase";  
Caenorhabditis elegans delta6-fatty-acid-desaturase mRNA, complete cds.  
Length = 443

Score = 79.2 bits (192), Expect = 4e-14

Identities = 100/390 (25%), Positives = 132/390 (33%), Gaps = 95/390 (24%)

Query: 41 KHPGGLNFLNLFIDEKQDLTEYFRTLH--SKQALKILKSFPKTGAKQE--ETESSKR--- 93  
KHPGG D T F H S QA K L K G E E + KR  
Sbjct: 28 KHPGGAVIEQY---RNSDATHIFHAFHEGSSQAYKQLDLLKKHGEHDEFLEKQLEKRLDK 84

Query: 94 -----FSILKKKLKH--LFEPNWPIEIGLFLTTFTLFVTGCLTQ 130  
F L++KL L + N + ++T ++ Q  
Sbjct: 85 VDINVSAYDVSVAQEKKMVESFEKLRQKLHDDGLMKANETYFLFKAISTLSIMAFAYLQ 144

Query: 131 K--WYF-SIPLLVLMQIISGWIGHSMNHNRR----NPILRKFAIVYAPLCGGFSNKKWWGRK 183  
WY S LL L GW+ H H + P+ +L + GFS WW K  
Sbjct: 145 YLGWYITSACLLALAWQQPGWLTHEFCHQOPTKNRPLNDTISLFFGNFLQGFSRDWWKDK 204

Query: 184 HNQHMMFTNNILKDEDI-----QHDKYKLWQFPFLF 213  
HN HH TN I D DI QH Y P L  
Sbjct: 205 HNTTHAATNVIDHDGIDIDLAPLFAFIPGDLCKYKASFEKAILKIVPYQHLYFTAMLPLMR 264

Query: 214 LKWKLDLSILASYE-----FEGIFLALHWVLLFNQNFYI-----VILS 251  
W S+ + E +E + HW +F Q F + I+S  
Sbjct: 265 FSWTGQSVQWVFKENQMEYKVYQRNFAWEQATIVGHWAWVFYQLFLLPTWPLRVAYFIIS 324

Query: 252 ELIAGFFSASILVGNHENEMKF--ERRITLPFFEHQIAASRNYAFHDIFSLIMGGMQYQ 309  
++ G A ++ NH + K+ RI F QI +RN L GG+ YQ  
Sbjct: 325 QMGGGLLIAHVVTFNHNSVDKYPANSRILNFAALQILTTTRNMTSPFIDWL-WGGLNYQ 383

Query: 310 TEHHFFPQIPFYRLPKARVIIAEELKKWNL 339  
EHH FP +P L + E K+ NL  
Sbjct: 384 IEHHLFPTMPRCNLNACVKYVKEWCKENNL 413

### Figure 3C

>trembl|U79010|BOU79010\_1 product: "delta 6 desaturase"; Borago officinalis delta 6 desaturase mRNA, complete cds. //:gp|U79010|2062403 product: "delta 6 desaturase"; Borago officinalis delta 6 desaturase mRNA, complete cds. Length = 448

Score = 67.1 bits (161), Expect = 2e-10  
Identities = 100/414 (24%), Positives = 154/414 (37%), Gaps = 100/414 (24%)

```

Query: 6   TQEEIVLENKPELLNEYKFIYKDTEYDCTEYAKSNKHPGGLNFLNLFIDEKQDLTEYFRT 65
          T +E+  +KP  +      +   YD +++ K   HPGG  L           Q++T+ F
Sbjct: 10  TSDELKNHDKP---GDLWISIQGKAYDVSDWVKD--HPGGSFPLKSLAG--QEVTDAFVA 62

Query: 66  LHSKQALKILKSFPKTGAKQEE---TESSK-----RFSILKKKLKHLFEPNWPIE 112
          H      K L F TG  ++  +E SK           +  +  KK  +F      I
Sbjct: 63  FHPASTWKNLDKF-FTGYYLKDYSEVSKDYRKLVEFEFSKMGLYDKKGHIMFATLCFIA 121

Query: 113 IGLFLTTF-TLFVTGCLTQKWYFSIPLLVLQMIIISGWIGHSMNHNHNR---NPILRKFAVY 168
          +  ++ +  LF G L      FS L+  + I SGWIGH  H      + L KF ++
Sbjct: 122 MLFAMSVYGVLFCEGVLVH--LFSGCLMGFLWIQSGWIGHDAGHYMVVSDSRLNKFMGIF 179

Query: 169 APLC-GGFSNKWWGRKHNQHHMFTNNILKDEDIQH----- 202
          A C G S WW      HN HH+  N++  D D+Q+
Sbjct: 180 AANCLSGISIGWWKWNHNAHHIACNSLEYDPLQYIPFLVSSKFFGSLTSHFYEKRLTF 239

Query: 203 -----DYKLWQFPFLFLKWKLDASILASY-----YEFEGIFLALHWVLL- 240
          Y+ W F +      +L+  + S           +E G +  W L
Sbjct: 240 DSLSRFFVSYQHWTFTYPIPCAARLNMYVQSLIMLLTKRNVSYRAHELLGCLVFSIWYPLL 299

Query: 241 -----FNQNFYIVILSELIAGF-----FSASILVGNHENEMKFERRITLPFFEHQ 285
          + +      VI S  + G           FS+S+ VG  +  FE++ T      +
Sbjct: 300 VSCLPNWGERIMFVIASLSVTGMQQVQFSLNHFSSSVYVGKPKGNWFEKQ-TDGTLDIS 358

Query: 286 IAASRNYAFHDIFSLLIMGGMQYQTEHHFFPQIPFYRLPKARVIIABELKKWNL 339
          ++ FH           GG+Q+Q EHH FP++P  L K      + E  KK NL
Sbjct: 359 CPPWMDW-FH-----GGLQFQIEHHLFPMPCNLRKISPYVIELCKKHNL 403

```

### Figure 3D

>tremblnew|AF126799|AF126799\_1 product: "delta-6 fatty acid desaturase";  
Homo sapiens delta-6 fatty acid desaturase mRNA, complete cds.  
//:gp|AF126799|4406528 product: "delta-6 fatty acid desaturase"; Homo  
sapiens delta-6 fatty acid desaturase mRNA, complete cds. Length = 444

Score = 63.6 bits (152), Expect = 2e-09

Identities = 92/390 (23%), Positives = 152/390 (38%), Gaps = 88/390 (22%)

```

Query: 31  YDCTEYAKSNKHPGGLNFLNLFIDEKQDLTEYFRTLHKSQAL--KILK-----SFPKTGA 83
          Y+ T++ S +HPGG + + E D T+ FR H K LK
Sbjct: 44  YNITKW--SIQHPGGQQRVIGHYAGE--DATDAFRAHPDLEFVGKFLKPLLIGELAPEEP 99

Query: 84  KQEETESSK---RFSILKKKLK--HLFEPNWPPIEIGLF-----LTTFTLFVTGCLTQ 130
          Q+ ++SK F L+K + +LF+ N + L + FT+F G
Sbjct: 100 SQDHGKNSKITEDFRALRKTAEDMNLFKTNHVFLLLLAHIIALESIAWFTVFYFGNGWI 159

Query: 131 KWFYSIPLLVLQMIIISGWIGHSMNH-----NRNPILRKFAVYAPLCGGFSNKKWGRK 183
          + +L Q +GW+ H H N ++ KF + + G S WW +
Sbjct: 160 PTLITAFVLATSQAQAGWLQHDYGHLSVYRKPKWNHLVHKFVIGHLK---GASANWWNHR 216

Query: 184 HNQHMMFTNNILKDEDIQ--HDYKL--WQFPFLFLKWKL-----DSIL 222
          H QHH N KD D+ H + L WQ P + K KL ++
Sbjct: 217 HFQHHAKPNIFHKDPDVNMLHVFVLGEWQ-PIEYGKKKLKYL PYNHQHEYFFLIGPPLLI 275

Query: 223 ASYYEFEGI-----FLALHWVLLFNQNFYIV-----ILSELIAGFFSASILVGNH- 267
          Y++++ I ++ L W + + F+I IL L+ F + + +H
Sbjct: 276 PMYFQYQIIMTMIVHKNNWVDLAWAVSYYIRFFITYIPFYGILGALL--FLNFIRFLESHW 333

Query: 268 -----ENEMKFERRITLPFFEHQIAASRNYA---FHDIFSLLIMGCMQYQTEHHFFP 316
          M+ ++ +F Q+ A+ N F+D FS G + +Q EHH FP
Sbjct: 334 FVWVTQMNHIVMEIDQEAYRDWFSSQLTATCNVEQSFFNDWFS----GHLNFQIEHHLFP 389

Query: 317 QIPFYRLPKARVIIAEELKKWNLKIHEGP 346
          +P + L K ++ K ++ E P+
Sbjct: 390 TMPRHNLHKIAPLVKSLCAKHGIEYQEKPL 419

```

Figure 3E

>swiss|Q08871|LLCD\_SYNY3 LINOLEOYL-COA DESATURASE (EC 1.14.99.25)  
 (DELTA(6)-DESATURASE).//:trembl|L11421|SSD6DS\_1 product: "delta-6-Desaturase"; Synechocystis sp. delta-6-Desaturase gene, complete cds.  
 //:trembl|D90914|SSD914\_112 gene: "des6"; product: "delta-6-Desaturase";  
 Synechocystis sp. PCC6803 complete genome, 16/27, 1991550-2137258.  
 //:pironly|S35157|S35157 Delta(6)-desaturase - Synechocystis  
 sp.//:gp|D90914|1653589 gene: "des6"; product: "delta-6-Desaturase";  
 Synechocystis sp. PCC6803 complete genome, 16/27, 1991550-2137258.  
 //:gp|L11421|349563 product: "delta-6-Desaturase"; Synechocystis sp. delta-6-Desaturase gene, complete cds. Length = 359

Score = 43.4 bits (100), Expect = 0.003

Identities = 63/288 (21%), Positives = 101/288 (34%), Gaps = 61/288 (21%)

Query: 120 FTLFVTGCLTQKWYFSIPLLVLQMIIISGWIGHSMNHN---NP-ILRKFAIVYAPLCGGF 175  
 F LF + + L + + S +GH NHN NP I R + Y + G  
 Sbjct: 57 FVLFAFVIFPVRLLGCMVLAIALAASFVNVGH DANHNAYSSNPHINRVLGMTYDFV--GL 114

Query: 176 SNKWWGRKHNQ-HHMFNTNNILKDEDIQHDYKLWQFPFL-----FLKWKLDASILAS 224  
 S+ W +HN HH +TN + D +I D + P F W L +  
 Sbjct: 115 SSFLWRYRHNYLHHTYTNILGH DVEIHGDGAVRMSPEQEHVGIYRFQQFYIWGLYLFIFP 174

Query: 225 YYEFEGIFLAL-----HWVLLFNQNFYIVILS-----ELIAGFFSASILVG 265  
 Y+ ++L L H + F +L L GF +L+G  
 Sbjct: 175 YWFLYDVYLVNLNKGKYHDHKIPPFQPLELASLLGIKLLWLGYVFGLEPLALGFSIPEVLIG 234

Query: 266 NHENEMKFERRI-TLPFFEHE-----QIAASRNYAFHDIFSL 300  
 M + + T+ H QI + N+A ++ F  
 Sbjct: 235 ASVTYMTYGIVVCTIFMLAHVLESTEFLLTPDGESGAIDDEWAICQIRTTANFATNNPFWN 294

Query: 301 LIMGGMQYQTEHHFFPQIPFYRLPKARVIIAEBELKKWNLKIHEGPIFE 348  
 GG+ +Q HH FP I P+ II + +++ ++ P F+  
 Sbjct: 295 WFCGGLNHQVTHHLFPNICHIIHPQLENI IKDVCQEFGEVYKVYPTFK 342

M.alpina	1	----	MAAAPSVRTFTTRAEVLNAEALNIEGCKKDAEAPELMI	IDNKVYDVREHVP--DHPGG
C.elegans	1	-----	--MVLREQE--HEPFIKIDQKWCOIDDVLR-SHPGG	
B.officinalis	1	-----	---MAAQIKKYITSDLEKNHDK---PGDLMI	SIQGKAYDVSDWVK--DHPGG
M.musculus	1	MGKGNGEGSTERQA	PMPTRFRWEETIQKHNLRTDRWLVIDRKVNVTKMSQ--RHPGG	
T.thermophila	1	-----	----MGVDKTQEELIVLENKPPELLNIYKF	LYKDTEYDCIENAKSNKHPGG

Species	100	91	91	105	92	
<i>M.alpina</i>	----	FAAEVR	-----	KRLTLFQSE	GGYDSSKAMAFKVSFNLC	INGLSTIVIAKW
<i>C.elegans</i>	91	NMGTFNISEKRS	QAQINKSFTDL	RMVR	AEGLMDGSPLE	YIRKL-----
<i>B.officinalis</i>	91	-----	KDYR	-----	KLVFEFSK	MGLMD-KKGHIMFAT-LCFIAMLFAMSVYCVL
<i>M.musculus</i>	105	-KSSQIT	EDFR	-----	AKKTAED	NNLFKTNHLEFFELL
<i>T.thermophila</i>	92	-----	KR	-----	FSILKKKL	KHLFEPNPIEL-----
						GLLTFITFTLEFVT

225 M.alpina 146 CQTSTLANVLSAALLGLFWQCCGWLANDFLHEHQVFQDRFMDLFGAFLG GVCQ-----  
C.elegans 146 LQYHTYY-LPSAILMGVAVQQLGWLIIHEFAHHQLFKNRYVNDLASYFVGNFL QVSHIFNN  
B.officinalis 133 FCEGVLVHLFSCQLMGFLMIQSGWIGCHDACHYMVVSDSRLNKFMCI FAANCL S-----  
M.musculus 155 GTGWIPT-LVTLAFVLT SQAAQAGWLQHDYCHLSVYKKSIMNHNHVHKFVIGHLK-----  
T.thermophila 126 GCLTQKW-YFSIPLVLMQIISGWIGHSNHNHNRPILR-----KFALVYAPLCG-----



# Replacement Sheet

Figure 4B

M.alpina	199	GFSSWVKDKHNTHHAAPNVHGEDPDIDTHPLLTWSEHALEMFS	D--VPDEELTRMWSRF
C.elegans	205	GFSSGKWKEQHNVEHAAATNVVGEDDLDLMPFYATVAEHLNNYS	---QDS---WVMT
B.officinalis	186	CISIGWKKWNHNAHHICNSLEYDDELOYPFLVSSKFFGSLISHFYEKRLTFDSLSRF	
M.musculus	207	CASANWNHRHFQHHAKPNIHFHKDPDIKSLHVFVLGEWQPLEYG	---KKK---LKYL
T.thermophila	174	GFSENKWWGRKHNQHHMFTNNILKDED	---I---

5

M.alpina	257	MVLNQTWEXFPILSFARLSWCLQSILFVLPNGQAHKPSGARVPISLVEQLSLAMHWTWYL	
C.elegans	256	LFRWQHVVHWTFFMLPFLRLSMLLOSIIIFVSQMP-THYYDYRN-TAIYEQVGLSIHWAWSL	
B.officinalis	246	FVSYQHWTFFPIVCAARLNMVQSLIMLTKR---NVSYRA---QELLGCLVFSIWYP	
M.musculus	258	PYNHQHHEYFELICPELLIPMYFOYQIIMTMS	---RR---DWVDLAWAISYYM
T.thermophila	201	---QHDYKLVQFPFLFKWKLDSIIASYEF	---EGIFLALHVVLLF

10

15

M.alpina	317	ATMFLFIKDPVNM--LVMFLVVSQAVCGNIIAIVFSINHNCGMPVISKEEAVDMDFFIKQII	
C.elegans	314	G-QLYELPDWSTR--HMFELVSHLVCGFILLSHVVTFFNHYSVEKFALSSNIMSNYACIQIM	
B.officinalis	298	L-LVSCIPNNGER--LMFVHIASLSVTG-MQQVQFSTINHFSSVYVCK-PKGNMWFEEKQTD	
M.musculus	305	RFFYTYIPFYGILGALVPLNFIRFLESWFVWVTQMNHLVMEILDLDH---	YRDMFSSQLA
T.thermophila	242	N-----QN--FYIHHISELIAGFFSASILVGNHHEENEMKFERR--ITLPEFHEHOIA	

20

M.alpina	375	TGRDVH-PGLFANWFTCGLNYQIEHHILFPPSMPRHNFSKIQPAVETLCKKYNVRYHTTGM	
C.elegans	371	TIRNMR-PGRFIDWLWCGGLNYQIEHHILFPPMPRHNLNTVMPLEVKFEAAANGLPYMVDDYF	
B.officinalis	353	GTLDIS-CPPWMDWFHGGLOFOIEHHILFPPKMPRCNLRKISPYVIELCKKHNLPPNYASF	
M.musculus	362	ATCNVE-QSFFNDWFSGHLNFQIEHHILFPPMPRHNLHKIAPVKSICAKHGIEYQEKPIIL	
T.thermophila	288	ASRNYAFHDI FSLILINGCMQYQIEHHIFFPQIPFYRLPKARVIAEELKKNLKIHEGPIF	

25

30

# Replacement Sheet

Figure 4C

M.alpina	434	ECTAEVFSRLNEVSKAASKMGKAQ	457
C.elegans	430	TGFWLEIEQFRNIANVAAKLTKKIA	454
B.officinalis	412	KANEMTILRTLRNTALQARDITKPLPKNLVWEALHTHG	448
M.musculus	421	RALIDIVSSLLKKSCELWLDAYLHK	444
T.thermophila	348	EKSHL	352

Stucture of delta-6-Desaturase Gene

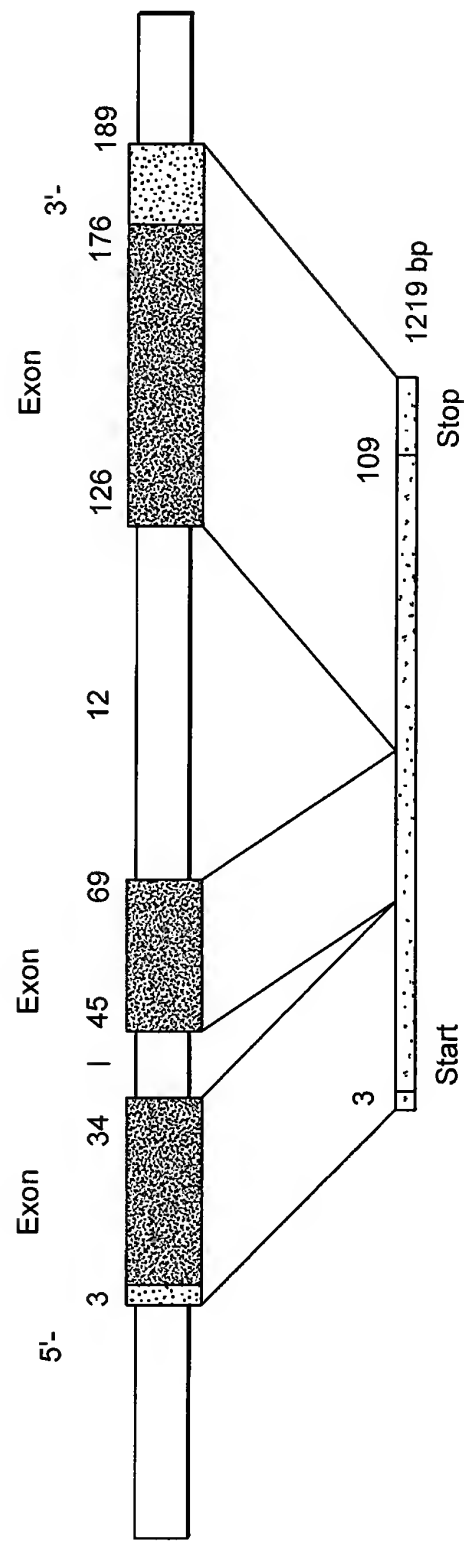


Figure 5

# Construction of pBDES6 Expressionconstruct

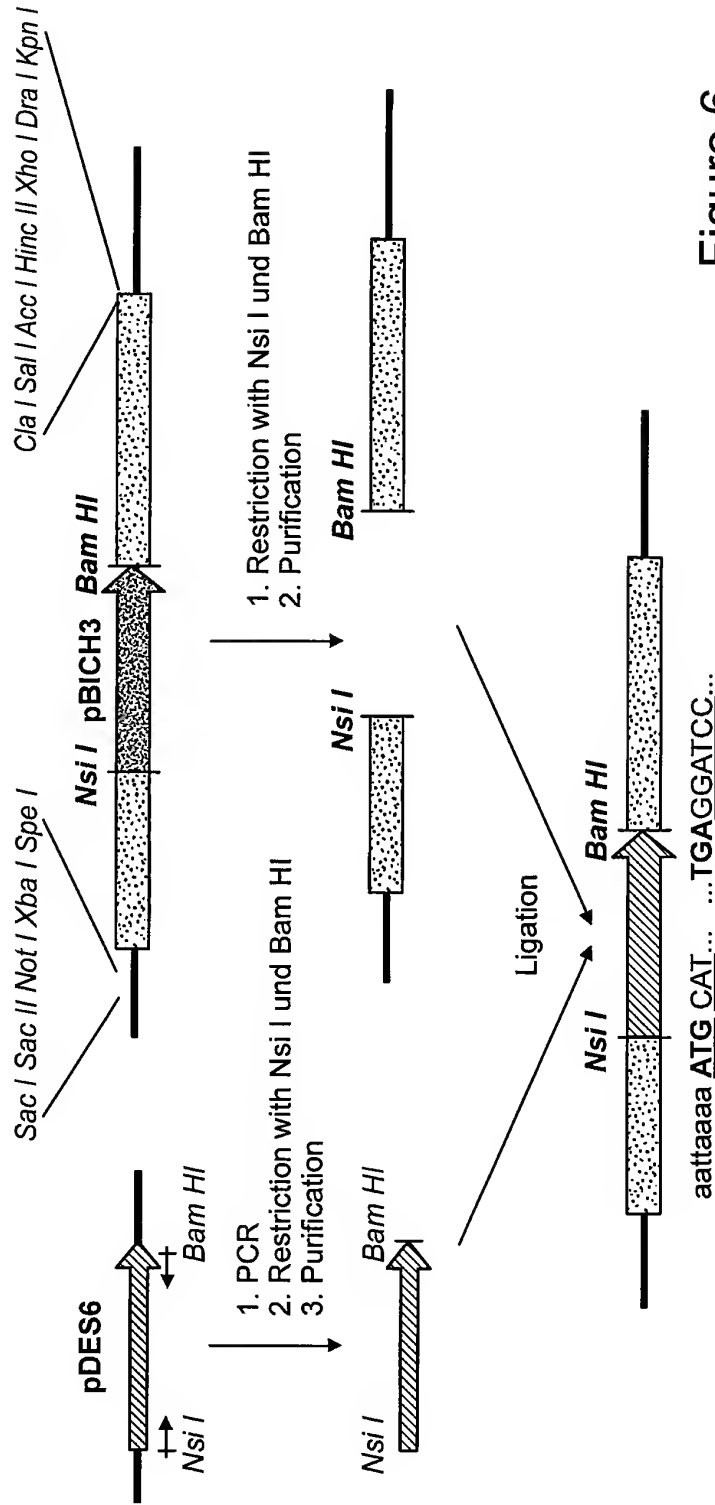
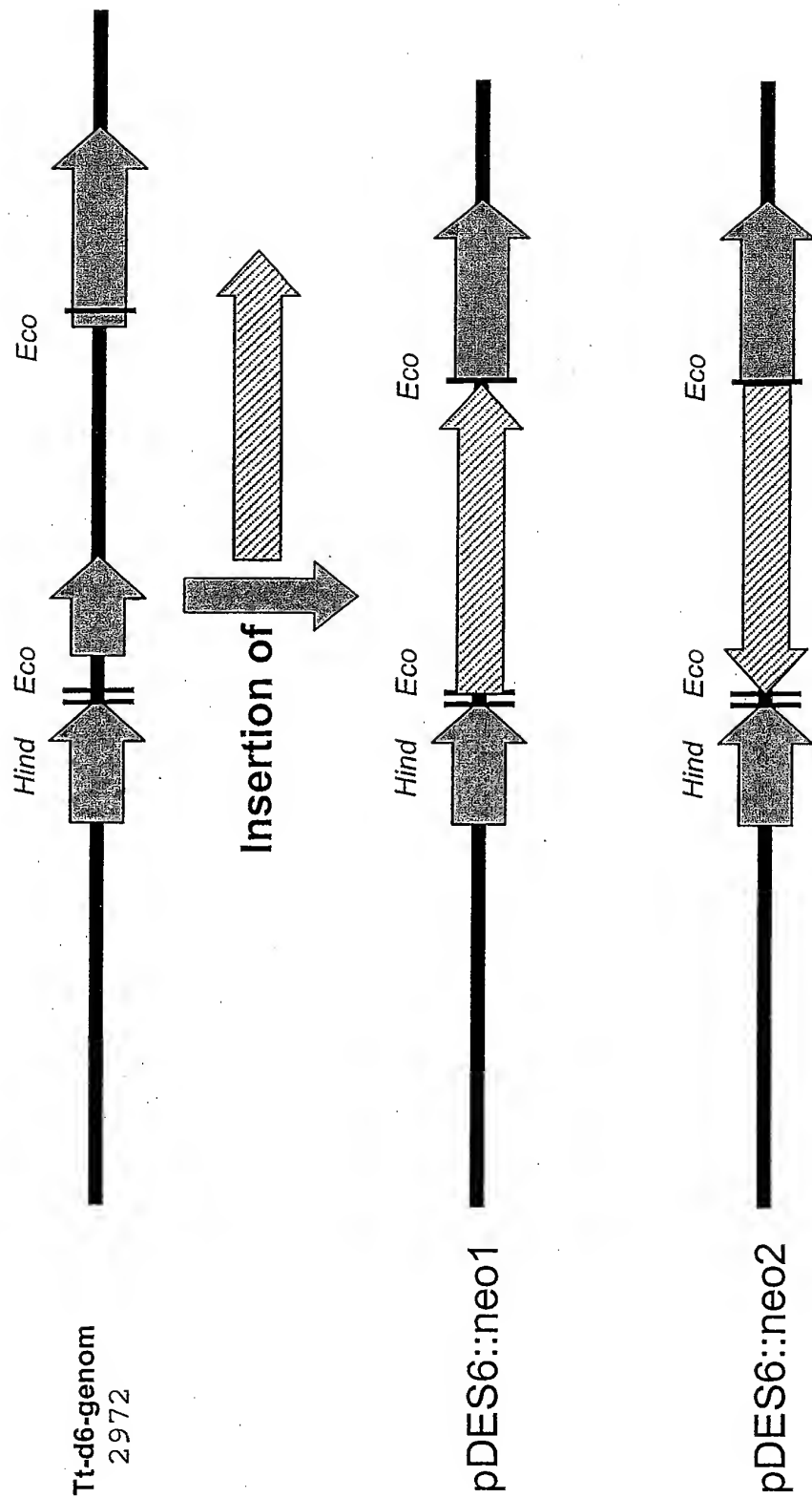
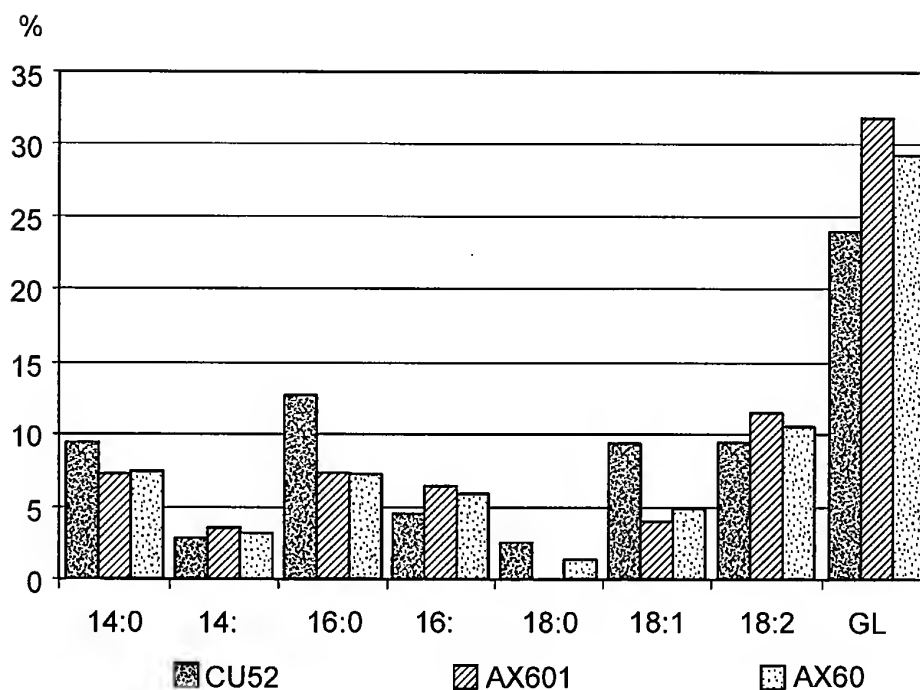


Figure 6

pBDes6 delta-6 Desaturase Expressionconstruct

Figure 7





Comparison of the fatty acid spectrum (main fatty acids) of the Tetrahymena pBDES6-Transformants (AX601 and AX604) with Tetrahymena wild strain (CU522) after 50 h of cultivation. Value is the percentage of fatty acids relative to total fatty acids.

Figure 8